

Exposure to Phthalate Esters

The American Chemistry Council Phthalate Esters Panel read with great interest the paper by Blount et al. (1) regarding urinary levels of phthalate ester metabolites in a sample set of the Third National Health and Nutrition Examination Survey (NHANES III) population. We have known for some time of this research, and have provided whatever information and assistance we could to the Centers for Disease Control and Prevention (CDC). The paper presents some interesting and informative data on the likely exposure levels of phthalate esters, which are summarized below:

- We believe that the data are analytically sound in that the CDC measured a physiologically relevant metabolite of the phthalate monoester as opposed to many investigators who have measured phthalate diester in biological samples (blood and tissues) as a biomonitor of exposure. Measurement of phthalate diesters in biological samples is highly subject to false positives from laboratory contamination.
- The CDC data measure urinary concentrations of phthalate monoesters, not the daily intake of phthalate esters. Although these measurements establish a good basis for biomonitoring, further calculations are needed to relate them to doses (below which we do not expect to see effects) developed from animal toxicology studies, such as U.S. Environmental Protection Agency (U.S. EPA) reference doses (RfDs), in order to place them into the risk assessment paradigm. Studies being conducted by the U.K. Ministry of Agriculture, Food, and Fisheries provide a key piece of information, namely, the molar ratio of phthalate ester metabolite in the urine of human volunteers given known amounts of phthalate ester (2). With this information, the CDC data can be converted to intake levels using the equation below by substituting the creatinine-corrected concentrations of monoester and a maximum output of 20 mg creatinine/kg/day for an adult female (range of 11–20 mg creatinine/kg/day) (3).

Daily intake (mg/ kg/ day)

= Urine concentration (mg/ g creatine)

↔ Creatine excretion (g/ kg/ day)

↔ MW diester (g/ mol)

÷ MW monoester (g/ mol)

↔ $\frac{\text{Monoester in urine (mol)}}{\text{Diester ingested (mol)}}$

- Using this conversion, the data indicate that the 95th percentile value of daily intake in all cases, except for diethyl phthalate

Table 1. Intake levels.

Phthalate	GM	95th Percentile	Highest value	Estimated intake	RfD
DEP	12.34 ^a	93.33	242.81	57 ^b	800
DBP	1.56	6.87	116.96	7	100
BBP	0.73	3.34	19.79	6	200
DEHP	0.60	3.05	38.48	30 ^c	20
DINP	0.21	1.08	14.35	10.8	ND

ND, not determined. ^aAll values in µg/kg/day based on a maximum creatinine clearance of 20 mg/kg/day. ^bEstimated intake taken from ATSDR, IPCS, or EU draft risk assessments. ^cFrom Doull et al. (4) using ATSDR estimates.

(DEP), is below the estimated intake values established by the Agency for Toxic Substances and Disease Registry (ATSDR; Atlanta, GA), the International Programme on Chemical Safety (IPCS; Geneva, Switzerland), or the European Union (EU) risk assessment as illustrated in Table 1 (95th percentile vs. estimated intake). Furthermore, the highest values obtained (including for DEP) are at or below levels that the U.S. EPA has determined to be safe for daily exposures to these phthalates (estimated intake vs. RfD).

- The highest value for total environmental exposure to the two most widely used plasticizers, di-(2-ethylhexyl) phthalate (DEHP) and di-isononyl phthalate (DINP), are at or below the levels estimated by various governmental agencies. Thus, environmental exposure to the two phthalate esters used in most polyvinyl chloride (PVC) consumer products (toys, shower curtains, etc.) is very low.
- The CDC data also show what appear to be higher levels of exposure of DEP, dibutyl phthalate (DBP), and butylbenzyl phthalate (BBP), a fact that has received attention. DEP, DBP, and BBP are used in some personal care products (6,7). Each of these phthalate esters also has Food and Drug Administration approved uses in food packaging and processing materials (8). Therefore, it is not surprising that urinary levels of these phthalate esters may be higher relative to those used only in PVC.
- As indicated by Blount et al. (1), all available pharmacokinetic data on phthalate esters indicate that they are rapidly metabolized and excreted from the body, and there is no concern that these materials bioaccumulate.

We recognize that the study reported by Blount et al. (1) is only a pilot project for a larger-scale biomonitoring program, and we look forward to the continued research in this area. We believe the CDC data demonstrate that environmental exposure to high molecular weight phthalates is negligible. For the low molecular weight phthalates, with potential exposures other than PVC plastics, the data indicate that exposures are within previously determined exposure estimates and below developed RfDs. Phthalate esters have been used for more than 50 years

(nearly 100 years in the case of DBP) without direct evidence of adverse human health effects. Although there are data for effects in rodents after exposure to phthalate esters, there are significant differences between rodents and primates in the pharmacokinetics and effects of these substances.

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REFERENCES AND NOTES

1. Blount BC, Silva MJ, Caudill SP, Needham LL, Pirkle JL, Sampson EJ, Lucier GW, Jackson RJ, Brock JW. Levels of seven urinary phthalate metabolites in a human reference population. *Environ Health Perspect* 108:979–982 (2000).
2. Anderson W, Ayesh R, Castle L, Scotter M, Springall C. A biomarker approach to quantify human dietary exposure to phthalates, risk assessment and communication for food safety [Abstract]. Presented at the First Joint CSL/JIFSAN Symposium on Food Safety & Nutrition, 20–22 June 2000, Central Science Laboratory, Sand Hutton, York, UK.
3. Tietz NW, ed. *Textbook of Clinical Chemistry*. Philadelphia, PA:W.B. Saunders Co., 1986:1821.
4. Doull J, Cattley R, Elcombe E, Lake BG, Swenberg J, Wilkinson C, Williams G, van Gemert M. A cancer risk assessment of di(2-ethylhexyl)phthalate: application of the new U.S. EPA risk assessment guidelines. *Regul Toxicol Pharmacol* 29:327–357 (1999).
5. Integrated Risk Information System (IRIS) database. Available <http://www.epa.gov/ngisgm3/iris> [cited 1990].
6. Brandt K. Cosmetic ingredient review on DEP and DBP. *J Am Coll Toxicol* 4:267–303 (1985).
7. Skinner JP. Final report on the safety assessment of butyl benzyl phthalate. *J Am Coll Toxicol* 11:1–23 (1992).
8. Food and Drug Administration, Department of Health and Human Services, 21 C.F.R. § 175–181, 1984.

Human Exposure Estimates for Phthalates

Phthalates are important industrial chemicals used in a variety of applications. These chemicals can be ingested, inhaled, or absorbed through the skin, resulting in human exposure and raising significant public health concerns.

Blount et al. (1) provide the first systematic compilation of data that address exposures of the general population to commercially important phthalate diesters. The data result from a study conducted at the Centers for Disease Control and Prevention/National Center for Environmental Health (CDC/NCEH) that is part of a continuing

collaboration with the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP). The study was designed to assess human tissue levels of potential or known environmental toxicants using biological samples collected through CDC's NHANES program and state-of-the-art analytical methods. Such data will aid the NTP in identifying chemicals in need of toxicologic evaluation, based on their prevalence in human tissues, as well as providing useful human exposure information on high priority toxic chemicals identified through the NTP or other toxicologic testing activities.

The primary route of human phthalate exposure to the general population has been presumed to be ingestion. Lower molecular weight phthalates can be absorbed percutaneously, and the more volatile congeners can be inhaled. Lipases in the intestinal epithelium, liver, and other tissues hydrolyze the diester to a monoester, which is systemically distributed. Some of the monoester is converted into other chemical species by oxidative metabolism in bodily tissues, and some of it is excreted in the urine as acyl glucuronides. The extent of oxidative metabolism and conjugation would be expected to vary among chemicals, species, and individuals. Blount et al. (1) report the concentrations of seven phthalate monoesters in the urine of 289 people. We present here the results of calculations of the estimated total daily intake of phthalates that would result in the reported urinary concentrations of monoester metabolites. These intake estimates can be used as a measure of total exposure to these phthalates and compared with previous intake estimates determined from levels in environmental media.

The daily exposure can be estimated by using a linear two-compartment model. The normalized integrated rate equations are

$$FE = 1 - \exp(-k_{total}t) \quad [1]$$

and

$$FU = \frac{k_u}{k_{total}} [1 - \exp(-k_{total}t)] \quad [2]$$

where FE and FU are the total and urinary fractions of the dose eliminated in time t , and k_{total} and k_u are the apparent first-order rate constants for total elimination and elimination of urinary monoester, respectively. The two rate constants were calculated from the excreted fractions observed during the 24 hr following a single oral dose of diester, using Equations 1 and 2.

Assuming steady-state intake and metabolic clearance of the diester, the internal exposure rate for an individual is approximated by

$$\begin{aligned} & \text{intake}(\mu\text{g} / \text{kg} / \text{day}) \\ &= \frac{ME(\mu\text{g} / \text{g}) \times CE(\text{mg} / \text{kg} / \text{day})}{f \times 1000(\text{mg} / \text{g})} \\ & \times \frac{MW_d}{MW_m} \end{aligned} \quad [3]$$

where ME is the urinary concentration of monoester per gram creatinine, CE is the creatinine excretion rate normalized by body weight, f is the ratio of urinary excretion to total elimination (k_u/k_{total}), and MW_d and MW_m are the molecular weights of the diesters and monoesters, respectively. We calculated the intake for each individual in the reference population using Equation 3. The presumed parent compound for urinary monoesters generally is the corresponding symmetrical diester. The presumed parent compound of urinary monobenzyl phthalate is *n*-butyl benzyl phthalate.

Published animal or human data for excretion of metabolites of di-*n*-butyl phthalate (2,3), *n*-butyl benzyl phthalate (4,5), di(2-ethylhexyl) phthalate (6,7), and di-*n*-octyl phthalate (8) allowed calculation of the fractional excretion values. The fraction of the dose of diethyl phthalate appearing as urinary monoethyl phthalate was assumed to be the same as for the di-*n*-butyl congener. The fraction of *n*-butyl benzyl phthalate excreted as benzyl phthalate was adjusted using the observation that this

monoester accounts for 64% of the urinary metabolites (9). The fraction found for di(2-ethylhexyl) phthalate was used for dicyclohexyl and di-*i*-nonyl phthalates as well because specific fractions could not be found for these diesters. The fractional excretion values used in our calculations are shown in Table 1. Creatinine excretion was set to 23 and 18 mg/kg/day for men and women, respectively (10).

The NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) conducts scientific evaluations of the literature on the reproductive and developmental toxicity of selected chemicals to which people are exposed. In Table 2, our intake estimates are compared to general population exposures estimated by the Phthalates Expert Panel of the CERHR based on published data. Because several phthalates have been shown to be developmental toxicants in laboratory studies, exposures of the 97 women of reproductive age (20–40 years) in the total sample and of the remaining individuals were calculated separately. These results are compared in Table 3. Excretion levels of the metabolites that were below the limit of detection (LOD; 1 ng analyte/mL urine) were assumed to be zero.

Uncertainties in the values of the parameters in Equations 1 and 2 are potential sources of error in our estimates of exposure. Creatinine excretion is known with 10% accuracy (10). FE is generally accurate

Table 1. Total fractional excretion (FE) and fractional urinary excretion of monoester (FU) during 24 hr after a single oral dose of diester.

Monoester	Diester	FE	FU
Ethyl	Diethyl	0.94 ^a	0.52 ^a
<i>n</i> -Butyl	Di- <i>n</i> -butyl	0.94 ^b	0.52 ^c
Benzyl	<i>n</i> -Butyl benzyl	0.70 ^d	0.36 ^e
Cyclohexyl	Dicyclohexyl	0.65 ^f	0.069 ^f
2-Ethylhexyl	Di(2-ethylhexyl)	0.65 ^g	0.069 ^h
<i>n</i> -Octyl	Di- <i>n</i> -octyl	0.65 ^f	0.043 ⁱ
<i>i</i> -Nonyl	Di- <i>i</i> -nonyl	0.65 ^f	0.069 ^f

^aAssumed to be the same as di-*n*-dibutyl phthalate. ^bData from Tanaka et al. (2). ^cData from Foster et al. (3). ^dData for the urinary fraction from Nativelle et al. (4); data for the fecal fraction from Eigenberg et al. (5). ^eAdjusted value from Nativelle et al. (4) for observed urine content (9). ^fAssumed to be the same as di(2-ethylhexyl) phthalate. ^gData for the urinary fraction from Peck and Albro (7); data for the fecal fraction from Kluwe (6). ^hData from Peck and Albro (7). ⁱData from Albro and Moore (8).

Table 2. Estimated exposures (mg/kg/day) to the general population based on extrapolated intake from urinary metabolites (Equation 1) in 289 individuals measured by Blount et al. (1).

Monoester	Diester	Minimum	Median	95th percentile	Maximum	CERHR ^a
Ethyl	Diethyl	< LOD	12	110	320	NA
<i>n</i> -Butyl	Di- <i>n</i> -butyl	0.084	1.5	7.2	110	2–10 ^b
Benzyl	<i>n</i> -Butyl benzyl	0.094	0.88	4.0	29	2 ^c
Cyclohexyl	Dicyclohexyl	< LOD	0.026	0.25	2.3	NA
2-Ethylhexyl	Di(2-ethylhexyl)	< LOD	0.71	3.6	46	3–30
<i>n</i> -Octyl	Di- <i>n</i> -octyl	< LOD	0.0096	0.96	13	< 3–< 30 ^d
<i>i</i> -Nonyl	Di- <i>i</i> -nonyl	< LOD	< LOD	1.7	22	< 3–< 30 ^d

^aThe CERHR Phthalates Expert Panel held its third and final meeting on 12–13 July 2000 in Arlington, Virginia; the CERHR final reports on the seven phthalates evaluated, along with a full description of the center and its activities, are available on the CERHR web site (11). ^bThe upper bound for occupational exposure was estimated as 286 μg/kg/day; the estimate of 2 μg/kg/day is at the 84th percentile of our calculated values. ^cThe CERHR estimate for *n*-butyl benzyl phthalate is at the 11th percentile of our calculated values. ^dDi-*n*-octyl and di-*i*-nonyl phthalate estimates from the CERHR were reported as less than for di(2-ethylhexyl) phthalate.

to approximately 50% (4,5). *FU* can vary 15-fold among species, with humans in the middle of the range (12). This variability is an order of magnitude higher than the reproducibility of the same measurement among laboratories (4–6,12). Owing to the lack of human excretion data, fractional excretion values for the rat were used for some congeners. Therefore, our exposure estimates are probably reliable within an order of magnitude.

Exposures for the general population, estimated by the CERHR Phthalates Expert Panel from published data, are in good agreement with our calculated human daily intake estimates based on CDC median values and presented in Table 2. However, the maximal values of excreted monoesters (1) indicate that some individual exposures are substantially higher than previously estimated for the general population.

Women of reproductive age appear to be exposed to higher levels of di-*n*-butyl phthalate than are the remainder of the study population. This is particularly evident in the 95th percentile column for *n*-butyl phthalate in Table 2, where the estimated exposure values for women 20–40 years of age are approximately 5 times greater than the corresponding values for the other 192 individuals in the study.

The data reported by Blount et al. (1) will certainly lead to further efforts to derive accurate estimates of human exposures based on urinary metabolite levels. In addition, their data lead to several questions that should be addressed in the immediate future; for example:

- What are the sources and circumstances of exposure that result in a higher urinary level of diethyl phthalate metabolites than of the other six phthalates studied?
- What is the evidence for reproductive and developmental toxicity of diethyl phthalate?
- What are the sources and circumstances of

exposure that result in some women of reproductive age having higher urinary levels of *n*-butyl phthalate than the remainder of the study population?

- At what levels are humans exposed to other phthalates not included in this study?

It is important that answers to these and related questions be pursued by public health agencies including the NIEHS/NTP.

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REFERENCES AND NOTES

- Blount BC, Silva MJ, Caudill SP, Needham LL, Pirkle JL, Sampson EJ, Lucier GW, Jackson RJ, Brock JW. Levels of seven urinary phthalate metabolites in a human reference population. *Environ Health Perspect* 108:979–982 (2000).
- Tanaka A, Matsumoto A, Yamaha T. Biochemical studies on phthalic esters. III. Metabolism of dibutyl phthalate (DBP) in animals. *Toxicology* 9:109–123 (1978).
- Foster PMD, Cook MW, Thomas LV, Walters DG, Gangolli SD. Differences in urinary metabolic profile from di-*n*-butyl phthalate-treated rats and hamsters. A possible explanation for species differences in susceptibility to testicular atrophy. *Drug Metab. Dispos* 11:59–61 (1983).
- Nativelle C, Picard K, Valentin I, Lhuguenot JC, Chagnon MC. Metabolism of *n*-butyl benzyl phthalate in the female Wistar rat. Identification of new metabolites. *Food Chem Toxicol* 37:905–917 (1999).
- Eigenberg DA, Bozigan HP, Carter DE. Distribution, excretion, and metabolism of butylbenzyl phthalate in the rat. *J Toxicol Environ Health* 17:445–456 (1986).
- Kluwe WM. Overview of phthalate ester pharmacokinetics in mammalian species. *Environ Health Perspect* 45:3–9 (1982).
- Peck CC, Albro PW. Toxic potential of the plasticizer di(2-ethylhexyl) phthalate in the context of its disposition and metabolism in primates and man. *Environ Health Perspect* 45:11–17 (1982).
- Albro PW, Moore B. Identification of the metabolites of simple phthalate diesters in rat urine. *J Chromatogr* 94:209–218 (1974).
- Castle L. Personal communication.
- Harper HA, Rodwell VW, Mayes PA. Review of Physiological Chemistry. Los Altos, CA: Lange Medical Publications, 1977.
- The National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR). Available: <http://cerhr.niehs.nih.gov> [cited 14 September 2000].
- Albro PW, Corbett JT, Schroeder JL, Jordan S, Matthews HB. Pharmacokinetics, interactions with macromolecules and species differences in metabolism of DEHP. *Environ Health Perspect* 45:19–25 (1982).

Antitumor Effects of THC

1-Trans-delta⁹-tetrahydrocannabinol (THC), the main active component of marijuana, has been shown to exhibit anticancer activity (1,2). Galve-Roperh et al. (1) reported that intratumoral administration of THC induces apoptosis of transformed neural cells in culture, and also induces a considerable regression of malignant gliomas in Wistar rats and in mice deficient in recombination activating gene 2. These authors suggest that their “results may provide the basis for a new therapeutic approach for the treatment of malignant gliomas.” Regarding this interesting finding, we believe it is important to highlight the previous National Toxicology Program’s long-term *in vivo* bioassay results that showed definite antitumor activity of THC (3,4). This also appears relevant to the current controversy, at least in the United States, regarding the use of marijuana in clinical medicine.

Experimentally, groups of 60–70 male and female rats were administered 0, 12.5, 25, or 50 mg THC/kg body weight (bw), and male and female mice were given 0, 125, 250, or 500 mg THC/kg bw in corn oil by gavage for 104–106 weeks (3,4). During this 2-year period, individual animal body weights were reduced compared to controls, although all groups consumed the same amounts of food. More importantly, survival in all THC groups of male and female rats was significantly greater than the controls. For mice, survival was comparable among groups except for the high-dose males. Clinical findings in the THC groups included lethargy followed by hyperactivity, convulsions, and seizures, which occurred typically during and immediately after dosing or handling.

In both rats and mice, no increased incidences of neoplasms were considered related to the administration of THC (3,4). In fact, for several organ systems the incidences of background tumors in these strains were actually reduced. The incidences of mammary gland fibroadenomas and uterine stromal

Table 3. Comparison of estimated exposures ($\mu\text{g}/\text{kg}/\text{day}$) to 97 women aged 20–40 years to the rest of the population (192 individuals)^a based on extrapolated intake from urinary metabolites (Equation 1) measured by Blount et al. (1).

Monoester	Diester	Minimum	Median	95th percentile	Maximum
Ethyl	Diethyl	0.90	13	90	170
		< LOD	11	130	320
<i>n</i> -Butyl	Di- <i>n</i> -butyl	0.24	1.7	32	113
		0.084	1.4	6.5	50
Benzyl	<i>n</i> -Butyl benzyl	0.094	1.2	4.5	7.8
		0.11	0.78	3.4	29
Cyclohexyl	Dicyclohexyl	< LOD	0.051	0.24	0.45
			0.012	0.25	2.3
2-Ethylhexyl	Di(2-ethylhexyl)	< LOD	0.71	3.8	10
			0.71	3.5	46
<i>n</i> -Octyl	Di- <i>n</i> -octyl	< LOD	< LOD	0.65	1.5
			0.015	1.0	13
<i>i</i> -Nonyl	Di- <i>i</i> -nonyl	< LOD	< LOD	3.7	7.8
				1.4	22

^aValues for women aged 20–40 years in boldface; remaining values are for the rest of population.

polyps were decreased in THC groups of female rats, as were incidences of pituitary gland adenomas, interstitial cell adenomas of the testis, and pancreatic adenomas in THC-treated male rats. Concerning nonneoplastic lesions in mice, increases of thyroid gland follicular cell hyperplasia occurred in all THC groups, and increases of forestomach hyperplasia and ulcers occurred in THC groups of male mice; yet, no THC-related tumors were observed to progress from these toxic lesions. This common lack of correlation between toxicity and carcinogenicity has long been known (5–7). Regarding carcinogenic activity of THC in mice, thyroid gland follicular cell adenomas were somewhat increased only in the lowest THC-dosed group of mice (125 mg/kg); thyroid gland follicular cell adenomas were found in 0/62 control males versus 6/60, 3/61, and 1/57 mice treated with 125, 250, and 500 mg THC/kg bw, respectively, and in 4/60 control females versus 9/60, 3/60, and 1/60 mice; these were considered not significantly related to THC (3,4). However, there were significant decreases observed for both benign and malignant liver tumors in male and female mice.

The reduced body weights in these long-term studies may have been contributory to the lowered tumor rates (8–10), as most of the reductions in tumor incidences occurred in hormone-controlled organs (11). This should not detract from the overall antitumor effects of THC observed in both sexes of these species and strains. Until further studies are accomplished, these reductions in tumor incidences in six organs should be considered caused by or associated with administration of THC.

Our 2-year studies (3,4) showed that the observed THC antitumor effects are not confined to the site of injection or administration, and these antitumor effects seem to affect a range of “spontaneous” tumors commonly found in rats and mice. Consequently, the THC-associated antitumor effects are systemically active and are applicable to different tumor types at different organ sites. Again, this lack of specificity might lend credence to the notion that these effects are hormonally mediated and likely related to the observed decreases in body weights. Nonetheless, there were significant reductions in total benign and malignant tumors in all organs combined for both species after THC exposure: in male rats, tumors were found in 98% of controls versus 98, 92, and 90% of groups treated with 12.5, 25, and 50 mg THC/kg bw, respectively; in female rats, tumors were found in 88% of controls versus 82, 86, and 70% of treated groups. Most strikingly, in male mice tumors were found in 73% of controls

versus 55, 44, and 30% of male mice treated with 0, 125, 250, and 500 mg THC/kg bw, respectively, and in female mice, tumors were found in 77% of controls versus 52, 43, and 27% of treated groups (3,4).

Interestingly, the dose levels used by Galve-Roperh et al. (1) were similar to those used in our studies (3,4). Their findings also agreed with ours in that THC administration did not affect either food or water intake or hematologic profiles and general clinical chemistry of the animals. Perhaps further animal bioassay studies should be done to learn more about the antitumor effects of THC. For example, animals could be exposed to known carcinogens (e.g., 9,10-dimethylbenz[*a*]anthracene exposure resulting in mammary gland tumors) to determine if THC would block this carcinogenic activity, or transgenic animals (12,13) could be used in an attempt to better clarify the mechanism(s) of THC anticarcinogenic activity. More definition of dose-response-antitumor activity relations would be useful, as would studies using paired feeding, to better define the influence of reduced body weight on tumor incidences.

With respect to genetic toxicology (4), THC was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535 with or without rat and hamster liver S9 fractions. In cultured Chinese hamster ovary (CHO) cells, THC induced sister chromatid exchanges at the highest dose tested in the presence of S9; at this dose level, cell cycle delay indicative of toxicity was observed. THC did not induce chromosomal aberrations in cultured CHO cells with or without S9 metabolic activation enzymes. *In vivo*, no increase in the frequency of micronucleated erythrocytes was observed in the peripheral blood of male or female mice administered THC by gavage for 13 weeks. Accordingly, THC does not appear to be genotoxic.

Long-term carcinogenesis bioassays have historically and traditionally been used primarily to identify those agents that cause cancer in laboratory animals and hence determine which agents represent a significant cancer risk to humans exposed to these carcinogens (14–16). Conversely, these bioassays can and should also be used to identify potential anticarcinogenic agents (17–19). Thus, in our studies, rats and mice that received THC for 2 years exhibited body weight reductions, enhanced survival rates, and decreased tumor incidences in several sites, mainly organs under hormonal control. These earlier experimental carcinogenesis results on THC (3,4) clearly lend further validity to the notion that cannabinoids may indeed be anticarcinogenic (1,2).

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REFERENCES AND NOTES

- Galve-Roperh I, Sanchez C, Cortes ML, del Pulgar TG, Izquierdo M, Guzman M. Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. *Nat Med* 6(3):313–319 (2000).
- Piomelli D. Pot of gold for glioma therapy. *Nat Med* 6(3):255–256 (2000).
- Chan PC, Sills RC, Braun AG, Haseman JK, Bucher JR. Toxicity and carcinogenicity of delta 9-tetrahydrocannabinol in Fischer rats and B6C3F1 mice. *Fundam Appl Toxicol* 30(1):109–117 (1996).
- NTP. Toxicology and Carcinogenesis Studies of 1-Trans-Delta⁹-Tetrahydrocannabinol (CAS No. 1972-08-3) in F344 Rats and B6C3F1 Mice (Gavage Studies). TR 446. Research Triangle Park, NC:National Toxicology Program, 1996.
- Hoel DG, Haseman JK, Hogan MD, Huff J, McConnell EE. The impact of toxicity on carcinogenicity studies: implications for risk assessment. *Carcinogenesis* 9(11):2045–2052 (1988).
- Huff J. Absence of morphologic correlation between chemical toxicity and chemical carcinogenesis. *Environ Health Perspect* 101(suppl 5):45–53 (1993).
- Huff J. Chemical toxicity and chemical carcinogenesis. Is there a causal connection? A comparative morphological evaluation of 1500 experiments. *IARC Sci Publ* 116:437–475 (1992).
- Rao GN, Piegorsch WW, Crawford DD, Edmondson J, Haseman JK. Influence of viral infections on body weight, survival, and tumor prevalence of B6C3F1 (C57BL/6N x C3H/HeN) mice in carcinogenicity studies. *Fundam Appl Toxicol* 13(1):156–164 (1989).
- Haseman JK, Young E, Eustis SL, Hailey JR. Body weight-tumor incidence correlations in long-term rodent carcinogenicity studies. *Toxicol Pathol* 25(3):256–263 (1997).
- Haseman JK. The National Toxicology Program experience with dietary restriction: does the manner in which reduced body weight is achieved affect tumor incidence? *Int J Toxicol* 17(suppl 2):119–134 (1998).
- Huff J, Boyd J, Barrett JC, eds. Cellular and molecular mechanisms of hormonal carcinogenesis: environmental influences. *Prog Clin Biol Res* 394: 1–479 (1996).
- Tennant R. Transgenic mouse models in chemical carcinogenesis studies. *Arch Toxicol Suppl* 16:261–270 (1994).
- Tennant RW, French JE, Spalding JW. Identifying chemical carcinogens and assessing potential risk in short-term bioassays using transgenic mouse models. *Environ Health Perspect* 103:942–950 (1995).
- Huff J. Long-term chemical carcinogenesis bioassays predict human cancer hazards. Issues, controversies, and uncertainties. *Ann N Y Acad Sci* 895:56–79 (1999).
- Huff J. Animal and human carcinogens [Letter]. *Environ Health Perspect* 107:A341–A342 (1999).
- Huff J. Value, validity, and historical development of carcinogenesis studies for predicting and confirming carcinogenic risks to humans. In: *Carcinogenicity Testing, Predicting, & Interpreting Chemical Effects* (Kitchin KT, ed). New York:Marcel Dekker, 1999:21–123.
- Douglas JF, Huff J, Peters AC. No evidence of carcinogenicity for L-ascorbic acid (vitamin C) in rodents. *J Toxicol Environ Health* 14(4):605–609 (1984).
- Chhabra RS, Huff JE, Haseman J, Hall A, Baskin G, Cowan M. Inhibition of some spontaneous tumors by 4-hexylresorcinol in F344/N rats and B6C3F1 mice. *Fundam Appl Toxicol* 11(4):685–690 (1988).
- Haseman JK, Johnson FM. Analysis of National Toxicology Program rodent bioassay data for anticarcinogenic effects. *Mutat Res* 350(1):131–141 (1996).